

FOR THE RECORD

Evidence for PDZ domains in bacteria, yeast, and plants

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Abstract: Several dozen signaling proteins are now known to contain 80–100 residue repeats, called PDZ (or DHR or GLGF) domains, several of which interact with the C-terminal tetrapeptide motifs X-Ser/Thr-X-Val-COO[−] of ion channels and/or receptors. PDZ domains have previously been noted only in mammals, flies, and worms, suggesting that the primordial PDZ domain arose relatively late in eukaryotic evolution. Here, techniques of sequence analysis—including local alignment, profile, and motif database searches—indicate that PDZ domain homologues are present in yeast, plants, and bacteria. It is suggested that two PDZ domains occur in bacterial high-temperature requirement A (htrA) and one in tail-specific protease (tsp) homologues, and that a yeast htrA homologue contains four PDZ domains. Sequence comparisons suggest that the spread of PDZ domains in these diverse organisms may have occurred via horizontal gene transfer. The known affinity of *Escherichia coli* tsp for C-terminal polypeptides is proposed to be mediated by its PDZ-like domain, in a similar manner to the binding of C-terminal polypeptides by animal PDZ domains.

Keywords: domain evolution; high-temperature requirement A; homology; intracellular signaling; tail-specific protease

A number of proteins associated with vertebrate tight or synaptic junctions in vertebrates or invertebrate septate junctions contain one or three imperfect copies of an 80–100-residue domain called the PDZ domain (Cho et al., 1992; Woods & Bryant, 1993; Kim, 1995). These proteins have been found as components of important sub-membranous structures and each contains a guanylate kinase-homologous domain; hence they have been termed membrane-associated guanylate kinases (MAGUKs). PDZ domains (previously called DHR or GLGF domains) also occur in other molecular contexts including protein kinases, phosphatases, a guanine nucleotide exchange factor (GEF) for Rac, neuronal nitric oxide synthase (nNOS), and syntrophins (Ponting & Phillips, 1995; Cho et al., 1992). To date, PDZ domains have been found only in invertebrate and vertebrate proteins.

Several MAGUK PDZ domains have been shown to bind a signature motif (X-Ser/Thr-X-Val-COO[−]) occurring as the C-terminal residues of K⁺ channels (Kim et al., 1995), NMDA receptor subunits (Kornau et al., 1995) and the adenomatous polyposis coli gene product (Matsumine et al., 1996). The second PDZ of a protein tyrosine phosphatase has been shown to bind Fas via a similar mechanism (Sato et al., 1995). However, not all ligands that bind PDZ domains do so via X-Ser/Thr-X-Val-COO[−] motifs. The third of five PDZ domains of *Drosophila InaD* appears to bind the Ca²⁺ channel trp via an internal (i.e., non-C-terminal) X-Ser/Thr-X-Val motif (Shieh & Zhu, 1996), and non-C-terminal PDZ-PDZ domain interactions have been identified involving nNOS and both PSD-95 and syntrophin (Brenman et al., 1996).

The crystal structures of liganded and unliganded PDZ domains have been determined recently (Doyle et al., 1996; Morais Cabral et al., 1996). These show a six β strand and 2 α helix structure that binds X-Ser/Thr-X-Val-COO[−] polypeptides via β -sheet augmentation (Doyle et al., 1996; Harrison, 1996). The structures show that various elements of PDZ sequences that are relatively well conserved (Ponting & Phillips, 1995) are important either for ligand-binding or for structural reasons. The loop linking β -strands A and B forms hydrogen bonds with the two carboxylate oxygens of the ligand, a conserved aspartic acid (strand β 4) forms a salt bridge with an arginine preceding β 1, and a conserved asparagine (β 4- α 2 linker) packs against the α 2- β 5 loop. The β 2- β 3 loop, whose length varies considerably in different domains, appears not to impede access of ligands to the binding site. PDZ domain sequences occur in many different vertebrate and invertebrate proteins indicating a widespread use of their fold and functions in multiple signaling pathways. Here, evidence is presented that PDZ-homologous domains occur in bacterial, plant, and yeast proteins and that these also possess C-terminal polypeptide binding functions.

Novel eukaryotic PDZ domains: Since our original report on PDZ domains (Ponting & Phillips, 1995) several PDZ domain-containing gene sequences have been deposited in databases (Fig. 1). Each of these has been identified using profiles (Birney et al., 1996) and/or motifs (Tatusov et al., 1994) and cross-checked using Blastp (Altschul et al., 1994) searches ($p < 1 \times 10^{-3}$ with previously-described PDZ domain sequences). These include 2 PDZs in a tyrosine kinase activator (TKA-1; K. Seedorf & A. Ullrich, unpublished, EMBO code Z50150) that also appears to regulate pro-

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Dlg1/Human-1	EYEEITLER.....	GNSGLGFSIAG	(10)	SSIFITKIITG.....	GAAAGDRLRVNDCLQVME	Dlg1/Human-1VVDVDRV...	TESKAVEALKEAGS...	IVRLVYKRRK	U13896	(221-311)	
Dlg1/Human-2	KIMEIKLIK.....	GPGLGFSIAG	(10)	NSIYVTKIEG.....	GAAHKDKLQIGDKLAVBN	Dlg1/Human-2VCLSEV...	TEEEAVTALINTSD...	FVYLVKAKPT	U13896	(316-406)	
Dlg1/Human-3	EPKRVVLR.....	GSTGLGFSIAG	(4)	EGIFISFILAG.....	GPADLSGELKGRDRISVBS	Dlg1/Human-3VCLRAA...	SEEQAAALNAGQ...	AVTIVAQYRP	U13896	(463-547)	
Inad/Dros-1	LIMVTLDR.....	TGKSFGLIIVR	(11)	TGIFIKGIVPD.....	SPAHLCGRLVGDRILSLVS	Inad/Dros-1KDVVNS...	TEQAVIDLKEADF...	KLELEIQTFD	U15803	(14-106)	
Inad/Dros-2	DLRIEIVQR.....	DASKFLGLALIA	(6)	MACFVAGVDPN.....	GALGVS.....	DIKPGDIEVBS	Inad/Dros-2NVLNKR...	CELNASAVFESVDDG...	KLVMITSRK	U15803	(246-333)
Inad/Dros-3	PKARIVQVR.....	KEGFLGIVIVY	(7)	SGIFISDLREG.....	SNALAG.....	KVKGDRILSLVS	Inad/Dros-3DVTLES...	NYDDATGLLEAEVGV...	VTHLLTLKSE	U15803	(362-450)
Inad/Dros-4	PNKKILIEL.....	KVEKPPMIVVC	(7)	TGCVITHVYFE.....	GQVAAKRLKIFDHICDIEG	Inad/Dros-4TPHVGSMITLKVHQLFHTTTE...	KAVTLTVFRAD	U15803	(484-576)		
Inad/Dros-5	EKFNDLMMK.....	AGKELGLSLSP	(2)	IGCTIADLIQG.....	QYPEIDSKLQRCITIKFSG	Inad/Dros-5DALEGL...	PFQVCYALFEGANG...	KVSMETVRPK	U15803	(581-664)	
Tks1/Human-1	RPRLCLVR.....	GEQGVYFHLG	(4)	RGQVIRRVPPG.....	SPAAAS.....	ALAGDRILVEBS	Tks1/Human-1VNVEGE...	THQVQVRIKAEVG...	QTRLLVVDQE	Z50150	(8-90)
Tks1/Human-2	RPRLCLVR.....	GEQGVYFHLG	(4)	RGQVIRRVPPG.....	SPAAAS.....	ALAGDRILVEBS	Tks1/Human-2QNVVGL...	RHAEVVASIKARED...	EARLLVVDPE	Z50150	(147-230)
Peri/Rat	ELVEIVET.....	EAQTGVSVFNVAG	(3)	EGIFVRELRD.....	SPAAKSLSLQEGDQLLSARV	Peri/RatFFENF...	KYEDALRLQCAEP...	YKVSFCLKRTV	Z29649	(15-100)	
Rhop/Mouse	LVGPVHTR.....	GEQGVYFHLG	(1)	SPVLIAAVVP.....	GQAESAG.....	LKEDYIVSVBS	Rhop/MouseQPCKW...	KLEVVTVLQSMGE...	EGVSLQVVSLL	U43194	(497-578)
Rit1/Rat	MTHAVTLR.....	GPSPVGFRLVG	(5)	APLTISRVAHAG.....	SKAALAA.....	LCQDSIAIAG	Rit1/RatESTELM...	TELEQNRKEGCHD...	HLTSLVSRPE	X76454	(1-84)
Clp3/Rat	MTQQIVLQ.....	GPSPVGFRLVG	(5)	QPLAISRVTPG.....	SKAALAN.....	LCIGDITAIID	Clp3/RatEDTSSM...	TELEQNRKEGCHD...	NMTLTVSRSE	U23769	(1-85)
Enig/Human	DSFKVYLE.....	GPAPVGFRLVG	(5)	VPLSISRLTPG.....	GKAQAQ.....	VAVGDVLSIDG	Enig/HumanENAGSL...	TELEQNRKEGCHD...	RLSGLSLRAQ	L35240	(2-85)
Apd/Human	GGRLVEVQLS.....	GGAPVGFRLVG	(5)	EPLVITKIEG.....	SKAAAVDKLLAGDIEVIGED	Apd/HumanICLSGF...	ROEALCLVSGSK...	TLKLVKRRS	X83543	(23-108)	
K147/Human-1	EELTLTILR.....	QTGGLGISIA	(11)	EGIFISRVSE.....	GPAAAG.....	VVGDVLSIDG	K147/Human-1VALQGA...	EHEAEVALLAGAGT...	AVQMRVVRER	D63481	(659-749)
K147/Human-2	QRVACLAR.....	SEGGVGFSLIAG	(11)	EGIFISRVSE.....	GPAAAG.....	VVGDVLSIDG	K147/Human-2VDVTEA...	REDHVSLLTAASP...	TALLLEDEA	D63481	(793-884)
K147/Human-3	PVEERLPR.....	AGGGLGISIA	(13)	PGVFSIKVLP.....	GAAARS.....	LVDGDRILAVEBS	K147/Human-3QDVVRA...	TEQAVSALLRPLC...	ELSLVLRPF	D63481	(935-1027)
K147/Human-4	GLRELQKKA.....	GPGLGFSIAG	(14)	EGIFISRVSE.....	GPAAAG.....	VVGDVLSIDG	K147/Human-4QSLGL...	TEGEVQLLSVGDG...	TLTVLVCDG	D63481	(1031-1126)
Pick/Mouse	VPGKVTLQKD.....	AQNLGISIAG	(5)	PCLYIVQVFN.....	TPAALD.....	ETVAADEITVSG	Pick/MouseKSIKKG...	TKVEVAKMIEVKG...	EVTHYTKLQ	Z46720	(19-105)
Prc_Bacul	TEMSLSL.....	SEIGAGVLM	(2)	DYTVNSVAG.....	GPAAKSKAIVGDKIVGVGG	Prc_BaculTGKPMVDVIGW...	RLDDVVALIKKPG...	KVRQELPAG	P23865	(239-323)	
Cupa/Syn3	VTTTGLL.....	SEIGAGVLM	(1)	NQLEIMAPLAG.....	SPAEAG.....	LQPHDQTLAIDG	Cupa/Syn3VDQTTL...	SLDEAARMKRPNT...	KVSLLEISA	L25250	(107-187)
Cupa/Spiol	VTGGSSL.....	SEIGAGVLM	(6)	TGLVVISATPG.....	SPAEAG.....	ILPQDVLIAIDG	Cupa/SpiolASTDKM...	GIYEALNAGVGG...	SVDTLICSRD	X90558	(227-310)
Cupa/Syn3	SQTSGLA.....	SEIGAGVLM	(4)	SDLVVDVMDG.....	TPALAK.....	IRPQDRIVRIG	Cupa/Syn3QPAALM...	SEGEATATQIGET...	ELSGLSLRPF	X96490	(135-215)
Cupa/Horu	KMSKYDM.....	TEIGLVIRE	(6)	IRLVVLGLILD.....	GPANAG.....	IRPQDRIVRIG	Cupa/HoruSDVRGK...	SAFDVSSMLQKPEK...	FVTIKVHKN	X90929	(55-138)
Cupa/Barba	RKSGEWF.....	GEIGLVIRE	(2)	NLIKVSPIDD.....	TPAAAG.....	VLAGDIEVSG	Cupa/BarbaKQISGQ...	TEQAVDQMRGPAET...	PPTLITNRP	L37094	(89-167)
Cupa/Bacu	ETISASF.....	SEIGAGVLM	(2)	GEILVSPKIG.....	SPAEAG.....	IKRPDQIKVNG	Cupa/BacuKSVKGM...	NVNEAVALLRKKET...	KVKELNLRAG	X98341	(97-135)
Htra/Pse-2	GAPGAERSS.....	NRLGVTVAD	(12)	GVVKEVQDQ.....	PAAVI.....	GLPQDVTILHND	Htra/Pse-2KAVTST...	KVFADVAKALPKNR...	SVSMRLVLRG	X32853	(375-463)
Htra_Ecoli-1	MVEYGGVQR.....	GEIGLVIRE	(14)	RGAFVSQVLPN.....	SSAAKA...	IKAGDVTILHND	Htra_Ecoli-1KPISSF...	AALRAQVGTMPVGS...	KLTGLLNDG	P09376	(280-371)
Htra_Ecoli-1	LIDFGEIKR.....	GLLGKIGTG	(14)	RGAFVSQVLPN.....	SSAAKA...	IKAGDVTILHND	Htra_Ecoli-1KPLNSF...	AEKLSRIATTEPST...	KVKGLVLRG	P39099	(258-349)
Htra_Ecoli-1	LIRDGRVIR.....	GYIGGGRE	(4)	GVVNVESPD.....	GPANAG...	IQVNDILISVDN	Htra_Ecoli-1KPAISA...	LETHDQVAILRPG...	VVPVVRGDD	P31137	(248-339)
Htra/Pse-1	LKAGKVS.....	GWLGVQIQE	(14)	SGALVAVQD.....	GPAAAG...	LQPHDQTLAIDG	Htra/Pse-1QSIRES...	ADPLHVLVNMPPG...	KINLGVIRNG	X32853	(261-352)
Htra/Barbe-1	LIEKGLVQR.....	GWLGVQIQE	(14)	KGALITDPLK.....	GPAAAG...	IKAGDVTILHND	Htra/Barbe-1EKINDV...	ROLAKRIANMSPG...	TVTGLVIRNG	L20127	(291-381)
Htra/Mycle	LIDKGVIVH.....	PTLVSTRS	(6)	SGALVANVAG.....	GPAAAG...	IKAGDVTILHND	Htra/MycleRKVADA...	DEFIVAVRLQTIQ...	DSEVVRGQ	U15180	(436-519)
Htra/Barbe-2	NMQDSKYS.....	NHSHSDET	(14)	LGVLVTVDPD.....	SDAAK...	IRPQDRIVRIG	Htra/Barbe-2KSVKVV...	SDITDTIKNAKGLR...	KAILQVIRNG	L20127	(398-490)
Htra_Ecoli-2	QVSSSIFN.....	GEIGLVIRE	(4)	GVVNVNKTG.....	TPAAQI...	LKKGIVTVIRNG	Htra_Ecoli-2QAVNKI...	AEKLVLDSPS...	VVALNIGED	P09376	(387-466)
Htra_Ecoli-2	SASAMITP.....	ALGATLSD	(7)	KGIKIDEVKG.....	SPAAAG...	LQPHDQTLAIDG	Htra_Ecoli-2DRVNSI...	AEKRVLAAPKA...	ILALQVIRNG	P39099	(365-447)
Htra/Human	AKGKAITKK.....	KYIGIRMS	(20)	SGAYIEVIPP.....	TPAAAG...	LKAGDVTILHND	Htra/HumanQSVVS...	ANDVSDIKRES...	TLMNVVRNG	D87258	(372-466)
Htra/Mypa	THIGPTAF.....	LGGLVTDNN	(1)	NGARVQVNT.....	GPAAAG...	IAQDVTIGVDT	Htra/MypaVPIING...	TSMTVLVPHPG...	DTIATVIRNG	Z23092	(267-344)
Htra/Yeast-1	GTIQVQWLLKPYDCRRRLGTSER	(11)	IGLVVAETVLR.....	EGPGYDK.....	IKEDGILHND	Htra/Yeast-1ETISSFMQVDKIQDENVGK...	ETQLVIRGQ	Z71399	(285-378)		
Htra/Yeast-2	CTVCTVGD(8)	YVVEGATFHE	(13)	RGVFLSSASGS.....	FNFDK.....	ERVGVIVRSDIN	Htra/Yeast-2KETPDL...	DTFIEIMKTIPTD...	RRKVTIVRYH	Z71399	(381-478)
Htra/Yeast-3	NGCKPVSII.....	VDAGFSGISVL	(20)	NRLQFTVSRV.....	SYTDKIH...	LETGDVILSVNG	Htra/Yeast-3KLVTEM...	NDLNGVSSADGILPSAMLDKVVVRG	Z71399	(751-854)	
Htra/Yeast-4	KIKTVEVQED.....	RFVIFAGSILQ	(13)	KGVYCTFRGES.....	SPALQV...	ISATNFITHVME	Htra/Yeast-4IETPDL...	DTFLKVVKTIPD...	NSYCKMLMT	Z71399	(860-953)
Sp4b_Bacu	VLPDLKVIP.....	GGQSIGVGLHS	(4)	VGFHQINTSGKKSPEGTAG...	IRAGDITIMNG	Sp4b_BacuOKIEK...	MNDVAPPIQAKGTG...	ESLDDLKIKR	P17896	(100-186)	
Sp4b/Clodi	NHDKFVYP.....	MGNIGVKNAT	(4)	DGVVLGYEKE.....	DVDYIG...	IQIGDNIVKIN	Sp4b/ClodiKRIKN...	SQVSEILNKES...	KVEVTFERN	U43514	(46-124)
Yael_Hacin	NLTNWFDPPEKESAFELGIMPNR	(2)	IRNVLSKVQW.....	SPAEKAG...	LQIGDKILKENT	Yael_HacinTALP...	WQDFIKQVEQGE...	SFSIKVIRNG	P49436	(195-276)	
Yael/Ecoli	DLRHAFEPDPEKEDPVSSLGIRPFG	(2)	IRNVLENQFN.....	SPAEKAG...	LQAGDRIVKVDG	Yael/EcoliQPLTQWTVFVMLVRDNPQK...	SLALIEIRGQ	D83536	(196-280)		

247y Structure	EEEE	EEEE	EEEE	EEEE	HHHH	EEEEEE
PDZ	eeee	eeee	eeee	eeee	hhhh	eeeee
asp-like	eeee	eeee	eeee	eeee	hhhh	eeeee
Asp1-like	eeee	eeee	eeee	eeee	hhhh	eeeee

Fig. 1. Multiple alignment of representative mammalian, *Drosophila* and *C. elegans* PDZ sequences, compared with tsp, htraA-like, yeast N1897 (HtrI/Yeast), SpoIVB (Sp4b) and Yael sequences. Residues that are conserved in $\geq 60\%$ of eukaryotic PDZ or tsp-like or htraA-like sequences are shown in outline. Hydrophobic residues (ACFILMVWY) conserved in $\geq 80\%$ (PDZ, tsp-like or htraA-like) or $\geq 75\%$ (HtrI/Yeast) sequences are shown in bold, as are residues absolutely conserved among known SpoIVB or Yael sequences. Secondary structure predictions (Rost & Sander, 1994) for htraA-like and tsp-like sequences are shown beneath the alignment, together with the known secondary structure of human Dlg PDZ3 (Morais Cabral et al., 1996). Numbers represent residues excised from sequences, and dots represent insertions/deletions. A methionine substituted (Met₄₄₂ → Lys) in a mutant form of *Drosophila* InaD that fails to bind the cation channel trp (Shieh & Zhu, 1996), is underlined. Domain limits and database accession codes follow the alignment. Species (in SwissProt format): Bacu, *Bacillus subtilis*; Barba, *Bartonella bacilliformis*; Barhe, *Bartonella henselae*; Clodi, *Clostridium difficile*; Drome, *Drosophila melanogaster*; Ecoli, *Escherichia coli*; Hacin, *Haemophilus influenzae*; Horvu, *Hordeum vulgare*; Human, *Homo sapiens*; Mouse, *Mus musculus*; Mycle, *Mycobacterium leprae*; Mycpa, *Mycobacterium paratuberculosis*; Pseae, *Pseudomonas aeruginosa*; Rat, *Rattus norvegicus*; Spiol, *Spinacia oleracea*; Syn3, *Synechocystis* sp. (strain PCC 6803); Yeast, *Saccharomyces cerevisiae*.

tein kinase A (Weinman et al., 1995), and single copies in rhophilin (Watanabe et al., 1996), periaxin (Gillespie et al., 1994), PICK1 (Staudinger et al., 1995), enigma (Wu & Gill, 1994), human APX-like protein (Schiaffino et al., 1995), CLP36 (Wang et al., 1995), and Rit18 (Wang et al., 1995). A human gene product (KIAA0147) contains leucine-rich repeats and 4 PDZs (Nagase et al., 1995). Several PDZs also occur in *Caenorhabditis elegans* putative proteins (Wilson et al., 1994), e.g., F25h2.2, T10a3.1, C01b7.4, T21c9.1, C45g9.7, F35d2.5, C01f6.6, and T19b10.5, and C52a11.4, which contain nine PDZ domains. The *Drosophila* inactivation no after-potential D (InaD) protein appears to contain 5 PDZs (Fig. 1) rather than the 2 originally reported (Shieh & Niemeyer et al., 1995).

Novel bacterial, yeast, and plant PDZs: The final iteration of a Swise database search (Birney et al., 1996; cf. Bork & Gibson, 1996)

used a profile derived from an alignment of 91 non-orthologous PDZ domain sequences. Surprisingly, 19 bacterial sequences scored higher (scores 4530–4694) than several previously-determined PDZ domain sequences (scores 4423–5182) and the perceived top “false positive” (score 4529). Using Blastp searches (Altschul et al., 1994), each of these 19 could be identified as a homologue of either of two *E. coli* periplasmic proteases: high-temperature requirement A (htraA; also known as DegP or protease Do) (Lipinska et al., 1989; Waller & Sauer, 1996), and tail-specific protease (tsp; also known as prc) (Hara et al., 1991; Silber et al., 1992). Homologues of bacterial htraA and tsp enzymes have been shown previously in humans (I. Ohno, J. Hashimoto, K. Takaoka, O. Takahiro, K. Okubo, K. Matsubara, unpublished, EMBO code D87258) and in higher plants (Oelmüller et al., 1996), respectively.

A variety of profile, motif, dotplot, and local similarity methods were subsequently employed to investigate whether the identification of htraA- and tsp-like sequences as PDZ domain candidates

was significant. Results from each of these methods (below) indicate that domains homologous to PDZ domains occur (a) in bacterial htrA- and tsp- homologues, (b) in bacterial stage IV sporulation B (spoIVB) and Yael proteins, (c) in the yeast htrA-like protein N1897, and (d) in plant tsp homologues.

Spread of PDZ domains via vertical or horizontal gene transfer?: Prior to this report PDZ domains were identified only in vertebrate and invertebrate proteins, making this distribution seemingly more limited than for other signaling domains such as src homology 2 or 3 (SH2, SH3) or pleckstrin homology (PH) domains, and suggests that the primordial PDZ arose relatively late in eukaryotic evolution. However, the identification of PDZ domains in bacterial, yeast, and plant proteins indicates either that the primordial PDZ domain arose prior to the divergence of bacteria or eukaryotes, or that horizontal gene transfer led to the acquisition of these domains by bacteria. It is noted that bacterial and human htrA-like sequences are considerably more similar to each other (percentage sequence identity $\approx 37\text{--}41\%$) than either is to each of the yeast htrA-like repeats ($\approx 19\text{--}27\%$). Indeed, the four yeast PDZ domains in the htrA-like molecule are barely detectable, and no further PDZ domains can be discerned in the complete *S. cerevisiae* genome sequence. Although conventional orthology between these sequences cannot be discounted, the strong sequence similarity between bacterial and mammalian PDZ domains is suggestive of a horizontal mode of transmission. It is noted that the non-PDZ region of tsps is sufficiently similar to mammalian interphotoreceptor retinoid-binding proteins (IRBPs) (Silber et al., 1992) to indicate that this also resulted from a horizontal gene transfer event between eukaryotic and bacterial genomes.

Functions of PDZ domains in htrA and tsp homologues: HtrA-like and tsp-like enzymes have not been suggested previously to contain homologous domains. However, it is notable that these periplasmic enzymes possess overlapping specificities that are required for growth or for protection from thermal and/or osmotic stress (Bass et al., 1996; Waller & Sauer, 1996). The active site of *E. coli* tsp is present in its IRBP homology region (Keiler & Sauer, 1995) and appears to be distinct from the C-terminal polypeptide binding site. The enzyme is known to cleave at discrete sites throughout polypeptide chains that possess C-terminal tripeptide sequences: $\phi\text{-}\phi\text{-}\sigma\text{-COO}^-$ (where ϕ and σ are non-polar, and small uncharged residues, respectively) (Keiler & Sauer, 1996). This specificity for C-terminal tripeptides is strikingly similar to the specificity of MAGUK PDZ domains for X-Ser/Thr-X-Val-COO⁻ motifs (Kornau et al., 1995). This suggests that metazoan PDZ domains and the PDZ-like region in bacterial tsp not only have evolved from a common ancestor but also possess a common function of binding C-terminal ligands. An extension to this argument predicts similar functions for other PDZ-like domains in bacterial proteins. These include PDZ domains in a small subset of enzymatically inactive htrA homologues that have been suggested to act as chaperones by binding denatured periplasmic proteins (Bass et al., 1996).

Detection of tandem PDZ-like repeats in htrA: Orthologues of htrA and an htrA-like protein (hhoA, or DegQ) were noted to contain two internal repeats in their C-terminal regions, likely to be a result of gene duplication; hhoB (or DegS) contains only one of these repeats. REPRO (Heringa & Argos, 1993) predicted two ≈ 88 amino acid tandem repeats in *E. coli* htrA (residues 292–379

and 396–474; score = 100). Importantly, these repeats in htrA and hhoA correspond to their PDZ domain-similar regions (cf. Ponting & Phillips, 1995).

Use of a local alignment method: Further evidence that the htrA-like repeats and regions of tsp proteases represent bacterial PDZ domains was obtained by performing Blastp searches (Altschul et al., 1994). For example, a search using the *Haemophilus influenzae* hhoA sequence produced a p -value of 1.2×10^{-5} when aligned with the PDZ regions of human KIAA0147, and the *Brucella abortus* tsp sequence yielded a p -value of 1.7×10^{-3} when aligned with the *C. elegans* F28f5.3 PDZ sequence. Similarly, *B. abortus* tsp and htrA sequences were found to be related by a p -value of 1.5×10^{-2} . Surprisingly, a *S. cerevisiae* ORF (N1897 gene product) produced a p -value of 2.0×10^{-9} when aligned with a *Pseudomonas aeruginosa* htrA homologue (mucD). Further investigation showed that this yeast hypothetical protein contains an internal duplication of an htrA-like sequence: the N-terminal repeat retains each of the catalytic triad residues of htrA serine proteases whereas these are all lacking in the C-terminal repeat (Fig. 2).

Use of motif, profile, and dotplot methods: In order to investigate the significance of these sequence similarities, the MoST algorithm (Tatusov et al., 1994) was used with initial PDZ $\beta 4$ strand-like alignment blocks of either (a) bacterial htrA-like repeat sequences (29 sequences), or (b) tsp-like sequences (9 sequences), or (c) known PDZ domain sequences (80 sequences). For these searches an expected/observed ratio $r < 5 \times 10^{-3}$ was chosen and all sequences that were aligned with p -values $< 10^{-5}$ were considered at the completion of the final iteration. Results demonstrated that, whichever starting sequence block was used, by the final iteration each of the three sequence classes (PDZ domains, tsp- and htrA-like sequences) were numerous represented with $p < 10^{-5}$. Pairwise alignments of PDZ and htrA, or PDZ and tsp, or htrA and tsp $\beta 4$ alignment blocks were all found to be significant (Z scores of 10.6, 8.8, and 18.1, respectively) using the method of Pietrokovski (1996).

Furthermore, for each of the three MoST searches three additional homology families were revealed with $p < 10^{-5}$. These were the yeast htrA-like hypothetical protein (N1897; see above), an *E. coli* hypothetical protein (Yael), and the *Bacillus subtilis* stage IV sporulation protein B (spoIVB) (Van Hoy & Hoch, 1990) (Fig. 3). Similarly, the highest scoring sequences in SWise searches using htrA- or tsp-derived profiles were PDZ domains, htrA-like

HtrI/Yeast-1	(112)	LGIIILTNREVV	(28)	DGFLKIDFP	(73)	GSNGSPVVNIDGYAVALQ	(328)	Z69382
HtrI/Yeast-2		RGYVLVSRRVV	(28)	NFAIVKYDP	(74)	CNNGILTDN-DGTVRGLW	(279)	Z69382
HtrA_Ecoli	(122)	RGYVVVNNREVV	(27)	DIALIQLQN	(64)	CNNGGALVNLNGELIGIN	(223)	P09376
Stp_Staau	(110)	KDTLLTNREVV	(39)	DIAIVKFSF	(65)	CNNGSPVFNERNVEVIGIS	(84)	P04188
Eta_Staau	(101)	KNTVLTNREVA	(45)	DIALIRLKF	(64)	CNNGSGTFVNSGELVGTN	(32)	P09331
<i>p</i> -values:								

Fig. 2. Alignment of regions of the yeast htrA-like hypothetical protein (HtrI/Yeast, N1897) repeats 1 and 2, with active site regions of *E. coli* htrA, *Staphylococcus aureus* V8 protease (sw: Stsp_Staau) and *Staphylococcus aureus* exfoliative toxin A (sw: Eta_Staau). Conserved His, Asp, and Ser residues that are essential for the serine protease activity of *E. coli* htrA (Skórko-Glonek et al., 1995) are shown in outline, and hydrophobic residues conserved in $\geq 80\%$ of sequences are shown in bold. Numbers represent amino acids that lie before, between, or after, alignment blocks. Calculated probabilities (p -values) (Schuler et al., 1991) of these alignments arising by chance are given beneath the alignment.

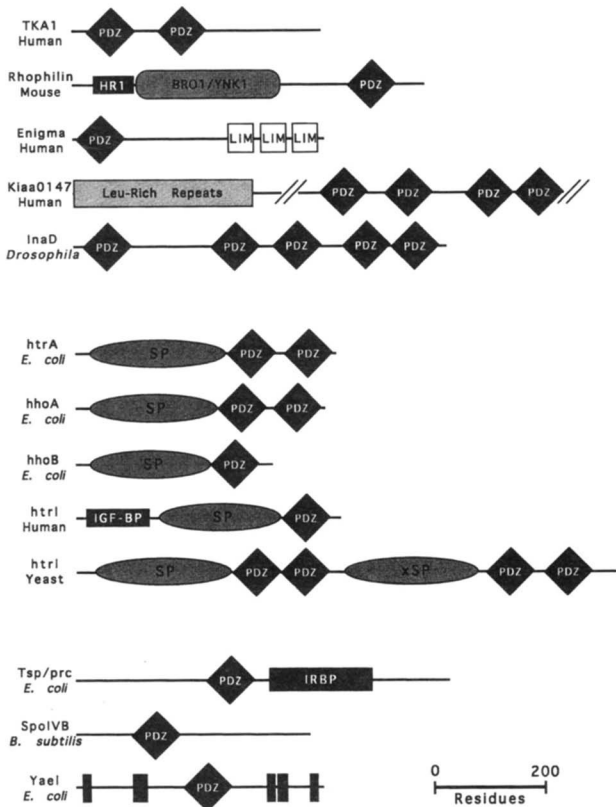


Fig. 3. Schematic representation of the domain organizations of PDZ domain-containing proteins (approximately to scale). Black boxes in Yael represent predicted transmembrane regions and double diagonals represent discontinuities in scale. Abbreviations: BRO1/YNK1, domain common to yeast Bro1p and to *C. elegans* R10e12.1 (Watanabe et al., 1996); HR1, homology region 1 Rho-binding domain; SP, serine protease domain (xSP, serine protease homologous domain, presumed to be inactive); IGF-BP, domain homologous to the insulin growth factor-binding protein; IRBP, domain homologous to interphotoreceptor retinoid-binding proteins.

repeats, and tsp, Yael, N1897, and spoIVB proteins (data not shown). Dotplots (Thompson et al., 1994) also demonstrated sequence similarities between PDZ and htrA-like repeats, and the presence of four htrA-like repeats in the yeast htrA homologue (not shown).

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